EMERGENCE OF LATERAL ROOTS[1]

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(WITH THREE FIGURES)

Our present conception of the method of emergence of lateral roots is based upon the elaborate exposition of the process made by VAN TIEGHEM in 1891. Since that time I am unable to find any record of emphatic disagreement with VAN TIEGHEM, though the results incidentally mentioned by later investigators of more or less related problems suggest the desirability of an examination of the evidence for his conclusion. Such an examination convinced me that we do not know whether the passage of the lateral root through the cortex is accomplished merely by mechanical pressure, or by a digestion of the cortical tissue, or by a combination of such methods.

It was my good fortune to be able to investigate this problem during the winter of 1907–1908 under the direction of Professor LUDWIG JOST at Bonn and at Strassburg. I am also indebted to the New York Botanical Garden for courtesies extended during the preparation of the manuscript.

Literature

After an extended anatomical study of the origin and emergence of lateral roots, VAN TIEGHEM[2] concluded that the young lateral root emerges by the dissolution of intervening tissue, and that this dissolution is accomplished by enzymes. In the case of the vascular cryptogams the meristematic pericycle is the secreting tissue, and in the case of the phanerogams this function is performed by the meristematic endodermis. His conception is that the young lateral root digests its way through the cortex just as an embryo digests its endosperm. Why VAN TIEGHEM was led to this conclusion is not clear, as he does not offer any substantial evidence, and I have been unable

1 From the botanical laboratory of the Kaiser Wilhelm University, Strassburg.

2 VAN TIEGHEM, PH., Traité de botanique. Deuxième édition. pp. 709–711. Paris. 1891.

to find in the numerous figures of VAN TIEGHEM and DOULIOT[3] any trace of corrosion of cell walls or actual evidence of digestion. The figures do not show the stratum of compressed and collapsed cells which surrounds the young lateral root, at least in the cases of *Lupinus albus* and *Vicia Faba.*

Before VAN TIEGHEM, the same general conclusion as to the digestive action of the lateral root was expressed by REINKE.[4] The figures of REINKE are more accurate than VAN TIEGHEM'S, as in *pl. 2, figs. 5, 9,* the layer of distorted and collapsed cells which surrounds the young lateral root is shown. Still the figures do not show actual evidence of digestion.

Earlier than VAN TIEGHEM but later than REINKE, VONHÖNE[5] undertook to ascertain the relative importance of mechanical pressure and enzyme activity in the emergence of endogenous organs. His conclusion is (p. 230) that the young lateral root secretes a substance which acts upon the cortical tissue and digests it, just as does the enzyme secreted by the embryo of a seed digest its endosperm.

PFEFFER[6] (p. 367) notes that the passage of lateral roots through the cortex may be purely mechanical, though aided perhaps by some correlative activity on the part of the cortical tissue.

PEIRCE,[7] in his study of the penetration of living tissue by the roots of Vicia and Pisum, found that the root mechanically pushes its way through the various tissues tested. The results of CZAPEK[8] also support PEIRCE, since the former was unable to find any evidence of diastatic or of inverting ferments in the excretion of the

3 VAN TIEGHEM, PH., ET DOULIOT, H., Recherches comparatives sur l'origine des membres endogènes dans les plantes vasculaires. Ann. Sci. Nat. Bot. VII. **8:** 1–660. *pls. 1–40.* 1888.

4 REINKE, JOHANNES, Untersuchungen über Wachsthumsgeschichte und Morphologie der Phanerogamen-Wurzel. HANSTEIN'S Bot. Abhand. Gebiet Morphol. und Physiol. **13:**1–38. 1871.

5 VONHÖNE, H. VON, Ueber das Hervorbrechen endogener Organe aus dem Mutterorgane. Flora **63:**227–234, 243–257, 268–274. 1880.

6 PFEFFER, W., Druck- und Arbeitleistung durch wachsende Pflanzen. Abhand. Königl. Sächs. Gesells. Wiss. **20:**235–474. 1893.

7 PEIRCE, GEO. J., Das Eindringen von Wurzeln in lebendige Gewebe. Bot. Zeit. **52:**169–176. 1894.

8 CZAPEK, FRIEDRICH, Zur Lehre der Wurzelausscheidungen. Jahrb. Wiss. Bot. **29:**321–390. 1896.

roots of higher plants. PEIRCE's conclusion is also supported by the results of OLUFSEN.[9]

Macroscopic study of the seedling

By examination of the seedlings of *Vicia Faba* one may find instances in which the cortex in the region of juncture of radicle and hypocotyl is strongly ruptured. In such cases one usually finds that two or more lateral roots have originated side by side, and their combined mechanical pressure has caused a rupture of the cortical tissue. The mere observation was made by VONHÖNE, but its significance apparently did not impress him. Evidently any digestion of the cortex that may occur is too slow to provide space for the advancing lateral root. Of course one cannot say positively that the fissure of the cortex is directly caused by the emerging lateral root. However, I have not found a rupture of the cortex except in association with lateral roots. Further, one may find swollen places on the radicle which are beyond doubt caused by the pressure of the advancing lateral root (*fig. 1*).

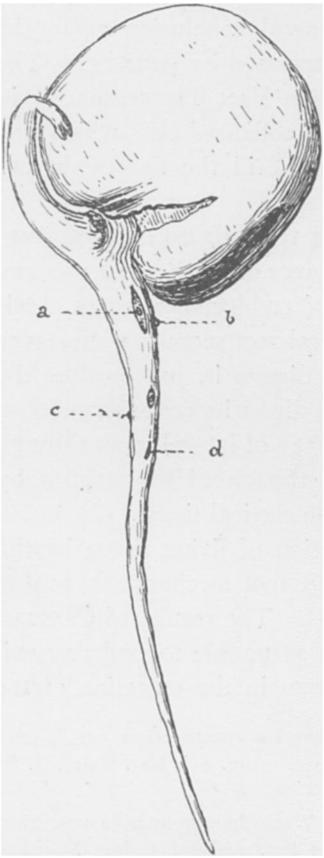

FIG. 1.—Seedling of *Vicia Faba:* *a*, rupture of cortex through which lateral root is emerging; *b*, c, cortex bulging because of pressure exerted by the lateral roots, which have not yet emerged; *d*, early rupture of cortex at point of emergence of lateral root.

Microscopic study of unemerged lateral root

All attempts to get microtome sections were unsuccessful, as the collapsed cells surrounding the young lateral root are lost in the preparation of the sections. All of my observations were

[9] OLUFSEN, LAURITS, Untersuchungen über Wundperidermbildung an Kartoffelknollen. Bot. Centralbl. Beih. 15:267–308. 1903.

made, therefore, from freehand sections mounted in pure lactic acid mixed with iodin. In order to have the sections clear and free from air, they were exhausted under the air pump. Only *Vicia Faba* and *Lupinus albus* were studied. Radial sections of the radicle showing the lateral root in median view are the best.

The cells of the cortex are not compressed to the stage of collapse until the lateral root has advanced about one-half the distance toward the epidermis. In earlier stages, when the lateral root has advanced only two-fifths the distance toward the cuticle, one may still find all the cells of the cortex uncollapsed and in natural cell connection, though of course displaced and compressed. The lateral root, therefore, has made a very difficult part of its journey without the slightest possibility of any digestion of the cortical cells. In the lupin the cells of the cortex have too little starch to note any possible autolysis, but in Vicia there is plenty of starch, and one can easily see that there is no difference in the starch content of the cells immediately surrounding the lateral root in comparison with those of other regions of the cortex. The same is true when the lateral root has advanced to the epidermis. Even in the cells which have been compressed to collapse, and in which the protoplasm looks wasted, the starch seems to be present in undiminished quantity. When the lateral root has advanced about one-half the distance toward the cuticle, the cell connection of the cells just outside the apex of the lateral root is broken in that region, and the cortical cells are thus pushed aside by the lateral root as is water by a boat. Those cells though now collapsed may remain undigested and be carried by the lateral root outside the epidermis. I have found, though very rarely, cases in which the cell connection of the displaced cortical cells was complete along the side of the lateral root, even at the time of the arrival of the latter at the epidermis. Any digestion of such cells is therefore excluded. Usually the cortical cells are so dislocated and so disarranged that the cell connection cannot be established. However, a few cases only are necessary to show that there can be no digestive action on the part of the lateral root either upon the cells of the cortex or of their contents.

Of course there must be some resorption of substance, and whether this is done by the cortex or by the lateral root I cannot say. As the

cortical cells collapse, the protoplasm of course loses its turgor and further compression drives the cell sap from the cell. Since the osmotic pressure of the lateral root is much higher than that of the cortical cells, there is probably some adjustment in the matter of resorption. Some of the solutes may go into the cortex and others into the lateral root. The apical cells of the lateral root are abundantly filled with starch, much more so than those of the cortex.

Turgor estimations

If the young lateral root mechanically pushes its way through the cortex, one would expect to find that the turgor of the cortical cells is less than that of the cells of the advancing lateral root. Longitudinal sections of the main root showing the lateral root in median view were immersed in various concentrations of potassium nitrate and of ammonium chlorid. After being exhausted under the air pump for thirty minutes, the degree of plasmolysis was determined. Essentially the same figures were obtained for *Vicia Faba* and for *Lupinus albus*. The figures given hold for sections in which the lateral root has nearly emerged. In 2 per cent. KNO_3 no plasmolysis could be observed in any of the tissues; in 3 per cent. KNO_3 there was initial plasmolysis of the cortical cells only. The turgor of the cells of the central cylinder and of those of the endodermis undisturbed by a lateral root seems to be a little higher than that of the cortical cells. In 4 per cent. KNO_3 the cortical cells are strongly plasmolysed, the endodermal cells covering the apex of the lateral root are not at all plasmolysed, the cells of the central cylinder and those of the undisturbed endodermis are somewhat though not strongly plasmolysed. In 5 per cent. KNO_3 there is total plasmolysis of all the cells except those of the lateral root itself and of the endodermal cells covering the apex of the lateral root. Some cells at the base of the lateral root show plasmolysis in 5 per cent. KNO_3. In 6 per cent. KNO_3 the endodermal cells covering the apex of the lateral root show initial plasmolysis. The maximum turgor for the endodermis is found in those cells which cover the apex of the young lateral root. From the apical cells toward the base of the lateral root the turgor of the endodermal cells seems to gradually decline, until only a short distance in longitudinal direction of the main root from the base of

the lateral root the turgor is the same as that of the undisturbed endodermis. The turgor of the cells of the central cylinder was also found to be 0.5 to 1 per cent. KNO_3 higher than that of the cells of the cortex. Similar estimates were made with ammonium chlorid, whose osmotic pressure is practically twice that of KNO_3, and it was found that one-half the concentration produced corresponding degrees of plasmolysis. In other sections in which the young lateral root is just beginning to dislocate the endodermis, the turgor of the endodermal cells is not so high, only about 4 per cent. KNO_3. It is thus apparent that as the very young lateral root commences to make new cells, the difference between the turgor of the cortical cells and that of the endodermal cells covering the apex of the lateral root increases until it amounts to about ten atmospheres before the lateral root ruptures the epidermis. *There can be no doubt that the tissue of the lateral root is capable of sustaining a growth push far greater than the cortex is capable of resisting.*

I was not able to observe plasmolysis in the meristematic cells at the apex of the lateral root inside the endodermis. It is quite likely that volume determinations would have revealed some shrinkage, but in saturated KNO_3 no separation of protoplasm from the cell wall was seen. It is possible that the protoplasm was suddenly killed. RHEINHARDT[10] found that the lateral roots of *Vicia Faba* will develop and grow in solutions of sufficient concentration to mortally plasmolyse the surrounding tissue.

The penetration of one living root by another

For the purpose of auxiliary evidence, several tests were made to ascertain whether one main or lateral root can penetrate another radicle. A seedling with radicle 6 or 7^{cm} in length was pinned to a sheet of cork through the cotyledons, and under the hypocotyl, 1 or 2^{cm} from the cotyledons, a small block of cork was placed to raise the hypocotyl. A root model of glass tubing was drawn out and vertically held upon the hypocotyl while gypsum was placed around the tube and the seedling. As soon as the gypsum was hard

[10] RHEINHARDT, M. O., Plasmolytische Studien zur Kenntniss des Wachstums der Zellmembranen. Sonderabdruck aus der Festschrift für Schwendener. pp. 41. *pl. 14.* Berlin. 1899.

the model was withdrawn, and into the canal was inserted the radicle of another seedling, so that the tip of the latter almost touched the horizontal hypocotyl (*fig. 2*). The inserted seedling was then almost entirely covered with gypsum, and when hard the preparation was placed in sawdust. The block of cork under the hypocotyl was of course withdrawn, and thus the side of the hypocotyl opposite the entering root was not covered with gypsum, and if the entering root penetrated the hypocotyl it could find exit unobstructed by gypsum.

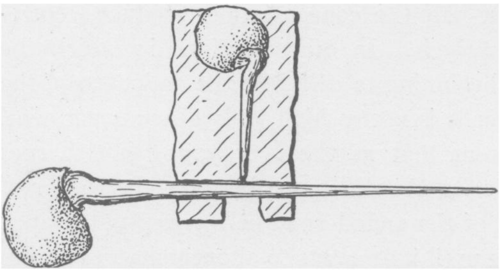

FIG. 2.—Arrangement of seedlings for penetration at right angles, the line-shaded portion representing solid gypsum.

Many such tests were made in which the main and lateral roots of *Vicia Faba*, *Lupinus albus*, and *Phaseolus multiflorus* were used in the various combinations, but the results were always negative. The preparations were allowed to stand undisturbed for various periods up to seven days, but the result was always negative. The radicle of the inserted seedling turned its tip as far as the canal would allow (less than the diameter of the radicle a mm. above the tip) and became imbedded in the callus subsequently formed. A strong imprint of the blunted radicle was always found on the hypocotyl, but microscopic examination showed the cuticle to be uncorroded and entirely intact. It is evident that if the radicle or the lateral root secretes an enzyme, such enzyme has no digestive action upon the cuticle. Apparently the mechanical push of the advancing root was not strong enough to break through the cuticle. However, no conclusion on this point is drawn here. It is significant, however, that in case the epidermis is ever so slightly wounded, as by piercing with a small glass point, either a main root or a lateral root will enter and pass entirely through the hypocotyl without formation of callus or other visible evidence of obstructed passage. Of course it is much easier to make an exit than an entrance through intact

cuticle if the latter is unwounded. When a root does enter, the passage is always around and not through the central cylinder, showing that the latter offers greater resistance than the cortex. Microscopic examination failed to reveal the slightest evidence of digestion of tissue. Many of the displaced cells of the cortex were found, but cells with broken walls were very scarce. The entering root apparently presses the cortical cells until they collapse, and then laterally displaces them without breaking the walls. No trace of corrosion of cell walls could be found. It seems probable that the mechanical push of the root is not sufficient to break through the cuticle when the latter is supported by underlying tissue. A demonstration for this, however, is not claimed.

Several tests were made to ascertain whether the central cylinder can be penetrated by another root at right angles. For this purpose enough cortex was removed to expose the stele, and the glass root model held so that when withdrawn from the gypsum the canal led directly to the central cylinder. In this way the entering root was compelled to enter the central cylinder at right angles or not at all. The result was negative in every case, and the entering root formed callus the same as in cases where the attempt was to enter the unwounded cuticle. The central cylinder was found to be strongly impressed, but no sign of corrosion or digestion of tissue could be observed. In some instances the entering root was able to shy from the stele and make passage through the cortex. Microscopic examination showed the cortex to contain compressed and collapsed cells. The appearance was the same as seen when the cortex is penetrated naturally by the lateral root. There was no evidence of corrosion, the walls of the collapsed cells being just as thick and regular in outline as those of the cortex in regions not affected by pressure.

Longitudinal passage through the stele by main and lateral roots

When the hypocotyl is traversed at right angles by another root, the tissue surrounding the path of the penetrating root cannot be examined microscopically with such advantage as is possible when the penetrating root traverses the hypocotyl longitudinally for a considerable distance.

The radicles of lupin seedlings having hypocotyls several centi-

meters long were amputated at the junction of stem and root. In the stele of the hypocotyl a vertical canal was made by inserting a small glass tube a few mm. and then withdrawing the tube. Into the canal was then inserted the root tip of a very young lupin seedling, and the whole preparation was then incased with gypsum and later placed in moist sawdust in vertical position (*fig. 3*). The parenchyma of the central cylinder offers the entering root the path of least resistance, so that the stele is often thus traversed its whole length. One may then make cross and longitudinal sections and very clearly study any changes in the tissues. As in the tests already described, no essential difference can be noted between the action of a main root and a lateral root on the tissues traversed by them.

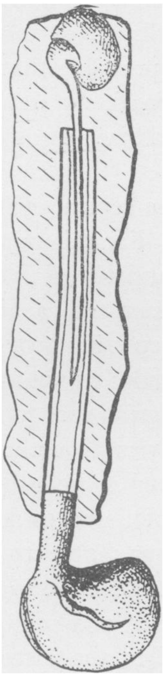

FIG. 3.—The radicle of the smaller seedling is growing downward through the central cylinder of the larger seedling's hypocotyl; the line-shaded portion represents solid gypsum.

The stele of the lupin is surrounded by a sheath which is only one cell in width, and the cells of this sheath only contain starch in any abundance. The cross-section thus stained with iodin shows the cylinder inclosed by a circle of starch-bearing cells. In cases in which the traversing root has pressed against this sheath, one may easily observe that even in cells of the sheath collapsed by the pressure the starch is present in undiminished quantity. There is no evidence of digestion of the starch either on the part of the entered root or by autolysis in the cells themselves. As one sections farther and farther along the hypocotyl, until only about 6 or 7^{mm} of the radicle remain within the hypocotyl, it may be observed, if the hypocotyl is allowed to remain undisturbed for a moment, that the radicle is gradually pressed out of the central cylinder, showing that it has encountered resistance from the tissues and that the latter recover from the pressure of the root. That the cells

of the central cylinder are under compression there can be no doubt, and that this compression provides some of the space occupied by the advancing root is also very clear. The collapse of some of the cells also provides space, and I am inclined to believe that in those two ways alone is the space occupied by the ingrowing root to be accounted for. Immediately surrounding the radicle, as seen in cross-section of the hypocotyl containing it, is to be found a stratum of collapsed cells which are so tightly compressed that one cannot count the individual cells. The walls are not corroded, however, and there is no evidence of wasting away of the tissue or of resorption of the cell walls. In some cells the protoplasm shows a little indication of wasting away, but it is too slight to count as a factor so far as the progress of the in-growing root is concerned. Beyond the cells which cannot be distinguished as separate units are those which can be so distinguished but which show compression without collapse. I have not in any case been able to account for each cell, but more than one-half the number can be found, which, together with those too much compressed to be distinguished, plus the space provided by compression, practically accounts for all the space occupied by the in-grown root. The compression is quite strong, as one may find cases in which the central cylinder is widely ruptured with the fissure extending for some distance into the cortex. Examination of the cells in immediate contact with the apex of the in-grown root shows them to be intact, so far as any corrosion or wasting-away of the walls is concerned.

Substitution of glass rod for the entering root

For the sake of greater certainty, preparations like the above were made except that a glass rod was vertically pushed into the central cylinder by a weight of from 300 to 400^{gm}. The rod was drawn out to resemble a root in form. After 48 hours under the weight the rod was removed and the tissues examined. The progress of the rod into the cylinder was somewhat slower than that of the growing root. The examination showed, however, no essential difference in the effect upon the tissues of the hypocotyl. One could not say from the microscopical examination whether the cylinder had been traversed by a root or by a glass rod. The compressed and collapsed cells had the same appearance as seen in other tests with root.

The passage of radicles and lateral roots through potato

Since PEIRCE found the penetration of living tissue by roots to be purely mechanical, I was not surprised at my failure to find sign of chemical activity on the part of penetrating roots in the tests above described. PEIRCE, however, found the radicle of Pisum able to enter the unwounded potato, a result which was difficult to understand, as I was inclined to assume the hypocotyl of Vicia and Lupinus to be more delicate than the epidermis or periderm of potato. A repetition of PEIRCE's experiment gave negative results. I tried both old and new potatoes, and also fitted glass tube tips to the advancing root to reduce deviation of the tip, but in each test the result was negative. Since PEIRCE used Pisum in his test, I tried that also, in addition to Lupinus and Vicia. If the periderm or epidermis is wounded, an easy entrance is effected by those radicles, and the root advances into the flesh of the potato. In tests with unwounded periderm there was always a deep impression of the potato. Microscopic examination showed that the periderm cells were compressed and to some extent the hypodermal tissue also, but there was no sign of corrosion. The advancing root formed callus, but when the periderm is wounded the callus does not form, and no evidence of obstructed passage is visible.

For the sake of another method a potato was cut into halves and the two halves tightly bound together with cord, so that the outside of one half was in contact with the outside of the other half. Perforations extending to within a few mm. of the periderm were made in one half, and into each perforation a seedling was inserted. The whole preparation was then incased with gypsum. Thus each radicle after penetrating a few mm. of the potato hypoderm found itself in contact with the inner side of the periderm. Further advance brought the tip of the radicle against the periderm of the other half of the potato from the outside. Strong impressions were made upon the periderm from outside. Microscopical examination showed no sign of any chemical activity. Since those same radicles readily traverse the flesh of the potato but do not enter the periderm from the outside, one is almost forced to the conclusion that the mechanical push is too weak. The advancing root simply follows the path of least resistance as long as an advance or deviation is possible. When

further advance is impossible, callus forms. Microscopical examination of the flesh of a potato through which a radicle has passed showed no evidence of any digestive action. The collapsed cells with uncorroded walls could be easily found, though the full number of cells required to occupy the volume of the tissue displaced by the radicle was not found in any given section. Compressed tissue too strongly compressed to allow a cell count was however present. No remnants of cells with frayed walls could be found. I did not see evidence of starch digestion in the immediate region through which the radicle passed. In many of the collapsed cells whose protoplasm appeared partially disintegrated, apparently intact starch grains were easily visible. There was no evidence of an active autolysis of starch grains in the cells.

Conclusion

The lateral roots of *Vicia Faba* and of *Lupinus albus* push out from the central cylinder through the cortex *mechanically* and do not have a digestive action upon the surrounding tissue.

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